Appendix A. Estimation of Coefficient of Variation

Let $z_k = \Delta \log R_k = \log[R_k / \mu_k]$, where R_k is the expression level of gene k from one of the fluorescent channels, and μ_k is the mean expression level of gene k in the same fluorescent detection channel (or from the same biological sample). We have the following approximation:

$$\frac{R_k}{\mu_k} = e^{z_k} \approx 1 + z_k + \frac{z_k^2}{2} \tag{A1}$$

or, $(R_k - \mu_k)/\mu_k \approx z_k + z_k^2/2$. Given the condition of constant coefficient of variation c provided in Eq. 6, c can be equivalently obtained by summing over all genes ($k = 1,\ldots, n$), instead of summing over multiple measurements of thesame expression level of gene k, given the fact that we only have one measurement for each gene, or $c_k^2 = E[(R_k - \mu_k)^2]/\mu_k^2 = (R_k - \mu_k)^2/\mu_k^2$. Then,

$$\begin{aligned} c^2 &= \frac{1}{n}\sum_{k=1}^{n} c_k^2 = \frac{1}{n}\sum_{k=1}^{n}\frac{(R_k - \mu_k)^2}{\mu_k^2} \\ &= \frac{1}{n}\sum_{k=1}^{n}(z_k + z_k^2/2)^2 = \frac{1}{n}\sum_{k=1}^{n}(z_k^2 + z_k^2 + z_k^4/4) \end{aligned} \tag{A2}$$

This approximation is good for $c < 0.3$. When $c < 0.15$, Eq. A2 can be further reduced to

$$c^2 = \frac{1}{n}\sum_{k=1}^{n} z_k^2 = \frac{1}{n}\sum_{k=1}^{n}\left(\Delta \log R_k\right)^2 = \sigma_{\log R_k}^2 \tag{A3}$$

Notice that we keep the subscript for the standard deviation annotation in Eq. A3, indicating that it is the variation of each gene even though they are the same, and not to be confused with the standard deviation of the gene expression levels which is defined by the biological system.

Gven that $y_k = \log R_k - \log R_k' = \log r_k$, let both R_k and R_k' be independent measurements of the gene expression level μ_{R_k}. Then, $\mu_{y_k} = 0$. Also,

$$\begin{aligned} y_k^2 &= \left[(\log R_k - \log \mu_{R_k}) - (\log R_k' - \log \mu_{R_k})\right]^2 \\ &= (\Delta \log R_k)^2 + (\Delta \log R_k')^2 - 2(\Delta \log R_k)(\Delta \log R_k') \end{aligned} \tag{A4}$$

Averaging over all expression levels, $k = 1,\ldots, n$,

$$\begin{aligned} \sigma_{\log r}^2 &= \frac{1}{n}\sum_{k=1}^{n} y_k^2 = \frac{1}{n}\left[\sum_{k=1}^{n}(\Delta \log R_k)^2 + \sum_{k=1}^{n}(\Delta \log R_k')^2\right] \\ &= \sigma_{\log R_k}^2 + \sigma_{\log R_k'}^2 \approx 2c \end{aligned} \tag{A5}$$

The sum over the second term in Eq. A4 is zero since R_k and R_k' are independent measurements.